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ORIGINAL RESEARCH PAPER

Simultaneous determination and pharmacokinetics studies of five isoflavones in rat plasma by UHPLC-MS/MS after oral administration of *Radix Astragali* extract

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ABSTRACT

A rapid, sensitive and convenient method based on ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) was developed and validated for the simultaneous quantification of calycosin-7-O- β -D-glucoside (CCSG), ononin, calycosin, (6aR,11aR)-9,10-dimethoxypterocarpan-3-O- β -D-glucopyranoside (DPPG), and 7,2'-dihydroxy-3',4'-dimethoxyisoflavan-7-O- β -D-glucopyranoside (DIFG) in rat plasma after oral administration of the methanol extraction from *Radix Astragali*. Theophylline played the role of internal standard (IS). Preparation of plasma samples by liquid-liquid extraction method with ethyl acetate after precipitation of protein with methanol. The analytes were detected with a triple quadrupole tandem mass spectrometry (MS) in multiple reaction monitoring (MRM) mode and a positive ion electrospray ionization (ESI). The method was validated with the concentration ranges of 1.96–62.69 ng/mL for CCSG, 1.70–54.5 ng/mL for ononin, 1.85–59.06 ng/mL for calycosin, 2.14–137.24 ng/mL for DPPG and 1.96–125.25 ng/mL for DIFG, respectively. The method had the lower limit of quantification (LLOQ) with 0.49, 0.21, 0.92, 1.07, and 0.98 ng/mL for CCSG, ononin, calycosin, DPPG and DIFG respectively, and the precision less than 10%. The RSD of the accuracy was in the range of –4.35–8.91%. The results may be helpful to provide more accurate references to clinical application of this herb.

KEYWORDS

Radix Astragali, UHPLC-MS/MS, isoflavones, rat plasma, pharmacokinetics

INTRODUCTION

Radix Astragali which is widely distributed in northwest and northeast China has been considered as a traditional Chinese medicine and health-care food for many years [1]. It is commonly used in numerous Chinese formulas to enhance “Qi” (vital energy). i.e., bu zhong yi qi decoction, gui pi decoction, yu ping feng powder, yangxin decoction, etc. [2, 3]. Isoflavone is a kind of metabolite of flavonoids in *Astragalus* [4], including calycosin-7-O- β -D-glucoside (CCSG), ononin, calycosin, (6aR,11aR)-9,10-dimethoxypterocarpan-3-O- β -D-glucopyranoside (DPPG), and 7,2'-dihydroxy-3',4'-dimethoxyisoflavan-7-O- β -D-glucopyranoside (DIFG) [5]. The pharmacological studies and clinical trials have demonstrated that these isoflavones possessed a lot of biological activities respectively benefiting antiviral activity, immunological competence, antioxidation activity and eliminating free radical, vaso-relaxation activity, hypoglycemic activity, inhibiting the increasing endothelial monolayer permeability [6–10]. They also played an important role in anti-osteoporosis, anti-fatigue, and anti-cancer activity, as well protecting the nervous system, removing the toxic

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metabolites, etc. [11–13]. Thus, in the research, the five isoflavones were chose as bioactive components for determination.

Several analytical techniques have been applied in the qualitative determination of CCSG, ononin, calycosin, DPPG, and DIFG, including electronic ionization mass spectrometry (EI-MS), high-performance liquid chromatography coupled with ultra-violet visible detector (HPLC-UV), LC-MS. Although these studies were mostly concentrated on the quantification of these isoflavones in raw materials or pharmaceutical forms, the simultaneous determination and pharmacokinetics of these five isoflavones have not been reported, especially no method has been used to compare the pharmacokinetics parameters of DIFG in plasma. The gastric emptying time and pH value in gastric fluid of rat were similar to human under a fasting state. Therefore, it was significant to establish a sensitive and simple method to simultaneous determination the five isoflavones in rat plasma. In addition, the method could be used for the comparison of the pharmacokinetic characteristics between CCSG and calycosin. In this study, we used ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) combining multiple reaction monitoring (MRM) technique for the simultaneous determination of five isoflavones in rat plasma.

MATERIALS AND METHODS

Herbal materials and reagents

R. Astragali was purchased from Shi Yi Tang Chinese Herbal Medicine Co. Ltd. of Harbin Pharmaceutical Group. The DPPG and DIFG were obtained from the *R. Astragali* by labotory previous isolation. CCSG (111920), calycosin (111530) and ononin were bought from the Chinese National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). The purity of five compounds was all higher than 98%. The chemical structures of the reference compounds were shown in Fig. 1. Acetonitrile, ethyl acetate, and methanol of HPLC grade were obtained from Merck

(Darmstadt, Germany) and distilled water was Watsons deionized water. Other chemicals were analytical grade.

Animals

Eight male SD rats, 230 ± 20 g, were taken from Shenyang Kangping Institute of Laboratory Animal (SCXK(Liao) 2014-0003) and were fasted for 12 h before administration with free water. Keep the animals in a dark cycle of 12 h/12 h under the conditions of ambient temperature 22–25 °C and relative humidity 60% (under 8:00 light). The experimental protocol was approved by the Animal Ethics Committee of Heilongjiang University of Chinese Medicine and conformed to the principles for the Care and Use of Laboratory Animals.

Instruments and conditions

The experiment was manipulated on an UPLC Acquity C₁₈ column (2.1 mm × 100 mm, 1.7 μm) maintaining at 40 °C. The mobile phases were consisted of solvent A (5 mmol/L ammonium acetate and 0.1% formic acid solution) and solvent B (acetonitrile) with a gradient elution of 5–50% (v/v) B at 0–1 min; 50% (v) B at 2–3 min; 50–5% (v/v) B at 3–4 min; 5% B at 4–5 min. The flow rate and injection volume were 0.3 mL/min and 2 μL, respectively.

Tandem mass spectrometry was carried out on an AB Sciex QTRAP™ 4000 mass spectrometer (AB Sciex, Foster, CA, USA) from Applied Biosystems controlled by Analysis 1.4 software (Applied Biosystems, Foster City, CA, USA) equipped with an ESI source worked in the positive ion mode. The parameters related to full scanning, product ion mass spectrometry and compounds were determined. Ion spray voltage (IS) = 4,500 V (all gases: nitrogen), ion source temperature (TEM) = 550 °C. The parameters of source and compound dependents were optimized as follows: curtain gas, 10 psi; ion source gas 1, 55 psi; ion source gas 2, 55 psi; The precursor-product ion transition was at m/z [447.2→285.2], [285.2→270.1], [431.1→269.2], [463.2→301.3], [482.3→303.2] and [181.2→124.1] for CCSG, calycosin, ononin, DPPG, DIFG and theophylline respectively. Quantification was performed using MRM and quantitative parameters were listed in Table 1.

Fig. 1. The chemical structures of analytes (1. DPPG; 2. ononin; 3. DIFG; 4. calycosin; 5. CCSG; 6. Theophylline)

Table 1. List of selected MRM parameters, declustering potential (DP) collision energy (CE), entrance potential (EP) and collision cell exit potential (EXP) for each analyte and IS

Analytes	Q1 Mass (Da)	Q3 Mass (Da)	DP (V)	CE(eV)	EP (V)	CXP (V)
DPPG	463.2	301.2	48.85	20.42	10.3	11.9
Ononin	431.3	269.1	64.95	22.21	9.5	10.2
DIFG	482.3	303.2	56.07	15.26	10.1	12.0
Calycosin	285.2	270.1	104.79	32.98	10.1	11.7
CCSG	447.2	285.2	69.02	23.60	9.9	10.2
Theophylline	181.2	124.1	62.21	27.22	10.6	12

Preparation of the extraction of *Radix Astragali*

Crude roots of *R. Astragali* (100.54 g) were crashed to coarse powder (60 mesh) and then extracted by methanol reflux for three times (3 h per time). After suction filtration and mixture of the obtained filtrate, the solution was concentrated with rotary evaporation under vacuum at 35 °C, which was lyophilized and finally obtained 8.55 g powder. The powder (40 mg) was weighed, dissolved in 2 mL methanol and analyzed by HPLC. The results showed that the contents of CCSG, ononin, calycosin, DPPG and DIFG were 0.968, 0.147, 0.237, 5.920, and 3.276 mg/g in the whole plants of *R. Astragali*.

Plasma sample Preparation

The following steps were the sample preparation processes: 100 µL rat plasma sample, 50 µL of IS (408 ng/mL in methanol), 100 µL methanol, and 1 mL ethyl acetate were mixed in 5 mL centrifuge tube and the mixed solutions was under ultrasound condition for 3 min and vortexed vigorously for 2 min. These samples were centrifuged at 3,500 rpm, 4 °C for 10 min. Finally, remove out the liquid and evaporate at 40 °C nitrogen until dry. The residues were redissolved with 100 µL 50% acetonitrile and vortex-mixed for 2 min. The processed samples were retransferred into 1.5 mL centrifuge tube and centrifuged at 12,000 rpm for 10 min.

Standard solutions, calibration curve and quality control (QC) samples

The mixture of stock standard solution containing CCSG (1.003 mg/mL), ononin (0.872 mg/mL), calycosin (0.945 mg/mL), DPPG (1.098 mg/mL) and DIFG (1.002 mg/mL) was intended by dissolving the accurate amounts of reference substances in methanol. Dilute the mixture solution with methanol to obtain working standard solution, whose concentrations were in the range of 0.98–62.69 ng/mL for CCSG, 0.43–54.50 ng/mL for ononin, 0.92–59.06 ng/mL for calycosin, 4.29–274.52 ng/mL for DPPG and 3.91–250.5 ng/mL for DIFG. The working concentration of IS was 408 ng/mL. All the stock working solutions were stored in the dark at 4 °C and reverted to room temperature before using. Six standard plasma samples for calibration curve were freshly prepared by evaporating to dryness 100 µL of the above working standard solutions and adding 100 µL drug-free plasma samples, the final concentrations remained constant. QC samples were independently prepared in the same way at low, medium and high concentrations: 3.918, 15.67, 62.68 ng/mL for CCSG, 3.410, 13.64, 54.56 ng/mL for ononin, 3.693, 14.77, 59.08 ng/mL for calycosin, 4.290, 17.16, 68.64 ng/mL for DPPG and 3.915, 15.66, 62.64 ng/mL for DIFG.

Method validation

The method was fully validated for selectivity and specificity, accuracy and precision, recovery and matrix effect, linearity and lower limits of quantification (LLOQ), and stability according to the lasted United States Food and Drug Administration (FDA) [14]. The selectivity was evaluated by comparing the chromatograms of blank plasma from six rats

with the plasma samples after oral administration. The precision and accuracy of method were assessed by performing replicate analyses of QC samples against calibration standards. The precision from inter-day and intra-day was determined by using six determinations of low, middle, and high concentration and expressed as relative standard deviation (RSD). The accuracy was judged by the relative error. Matrix effect was evaluated by comparing the peak areas of the analytes in the extracted blank rat plasma matrix to those of corresponding solutions at the same concentration. The extraction recovery was determined by calculating the ratio of the amounts of QC samples finally obtained against those originally spiked in the drug-free plasma. The freeze-thaw stability was evaluated by analyzing QC samples at three concentrations after undergoing for freeze (–80 °C) and thaw (room temperature) cycles in 1 month. The linearity of each calibration curve was determined by plotting the peak area ratio (y) of the analyte to the I.S. against the nominal concentration (x) of the analyte with weighted ($1/x^2$) least square linear regression. The LLOQ was determined as the lowest concentration point of the calibration curve at which the measured precision, expressed as the relative standard deviation (RSD). The calibration curves of five analytes ($S/N \geq 10$) could be quantified with RSD not exceeding 20%.

Application of the method and Pharmacokinetic study

Total eight rats were used for the pharmacokinetic experiments. This validated method was applied to detect the plasma concentrations of CCSG, ononin, calycosin, DPPG, and DIFG in rats after oral administration of the whole *R. Astragali* at a dose of 30 g/kg body weight (equivalent to 2.46, 0.36, 0.60, 15.00, and 8.31 mg/kg of CCSG, ononin, calycosin, DPPG and DIFG, respectively). Blood (0.2–0.3 mL) was collected from rat ophthalmic venous plexus at 0, 0.083, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h after oral administration of ERA. Within 30 min after blood withdrawal, the samples were centrifuged at 3,500 rpm for 10 min, then the plasma samples were separated and stored in Eppendorf Tubes at –20 °C prior to analysis.

Data analysis

Pharmacokinetic parameters analysis was performed by Drug and Statistics (DAS) 2.1 Software Program (Chinese Society of Mathematical, Pharmacology, Shanghai, China). Non-compartmental model was employed to calculate the following PK parameters: including area under concentration–time curve (AUC), maximum plasma concentration (C_{max}), time to reach the maximum concentrations (T_{max}), oral clearance (CL/F), and the half time ($T_{1/2}$).

RESULT AND DISCUSSION

Chromatographic condition

Acetonitrile, methanol, formic acid and ammonium acetate in water were tested to select the best mobile phase combination.

Acetonitrile, 5 mmol/L ammonium acetate and 0.1% formic acid solution were the final result. Furthermore, the addition of ammonium acetate solved the problem that the response signal of DIFG was low. Under the optimized conditions, the analytes and IS peaks were in well shape, with shorter running time, no obvious endogenous substances and no crosstalk (Fig. 3). We performed on an ACQUITY UPLC[®] BEH C₁₈ column (2.1 × 100 mm, 1.7 μm) and an ACQUITY UPLC[®] BEH amide column (2.1 mm × 100 mm, 1.7 μm), respectively. The results showed that the ACQUITY UPLC[®] BEH C₁₈ column had a good peak shape with a higher response value.

Optimization of MS conditions

For MS conditions, positive and negative ion models were verified with various mobile phase combinations. The results indicated that positive ion mode exhibited a higher response value of ions than that of negative ion models. The full-scan product ion spectra of analytes and IS were shown in Fig. 2.

Internal standard selection

In the experiment, puerarin, tramadol, rutin and theophylline were selected as internal standards. It was found that the response value of puerarin and tramadol was lower, and the rutin displayed had interference in plasma matrix. Finally,

theophylline was chosen as the internal standard because of the shorter retention time, stable response, good peak shape, effective separation, and no obvious interference in matrix.

Establishment of sample preparation method

At present, plasma treatment methods mainly included protein precipitation, liquid–liquid extraction, solid–liquid extraction and so on [15–18]. In the study, methanol, acetonitrile, ethyl acetate, diethyl ether, dichloromethane, acetone, and chloroform were served as the solvent for sample preparation. Finally, we decided plasma was precipitated with 100 μL methanol and then extracted with 1 ml ethyl acetate. There would be less interference and acceptable recovery rate by using the mixture solvents instead of a single solvent.

Method validation

Selectivity. Figure 3 showed the chromatographic profiles of blank plasma, blank plasma sample spiked with the five analytes at the LLOQ and IS, blank plasma was added to at QC samples and IS with five analytes, and plasma acquired 15 min after oral administration of *R. Astragali*. The retention time of CCSG, ononin, calycosin, DPPG, DIFG

Fig. 2. Full-scan product ion spectra of (a) theophylline (IS), (b) ononin, (c) CCSG, (d) calycosin, (e) DPPG, (f) DIFG in the positive ionization mode

Fig. 3. Typical MRM chromatograms of IS (1), ononin (2), CCSG (3), calycosin (4), DPPG (5) and DIFG (d). (A) blank plasma, (B) blank plasma sample spiked with the five analytes at the LLOQ and IS, (C) blank plasma sample spiked with the five analytes at QC samples and IS (54.50 ng/mL for ononin, 62.69 ng/mL for CCSG, 59.06 ng/mL for calycosin, 68.63 ng/mL for DPPG and 62.63 ng/mL for DIFG); (D) rat plasma samples collected 20 min after oral administration of ERA

and IS were 2.70, 2.96, 3.16, 3.03, 3.04, and 2.44 min. The total running time was 5 min per sample. No interfering peaks were detected at these retention times.

Recovery and matrix effects. The extraction recoveries of CCSG, ononin, calycosin, DPPG, DIFG and IS from rat plasma were shown in Table 2. The extraction recoveries of six repeated rat plasma containing five compounds with low, medium and high concentrations were determined. The mean recovery of the analytes was 76.38–113.9% (RSD < 8.35%), the corresponding matrix effect ranged from 91.39

to 99.21% (RSD < 7.79%). The extraction recovery and matrix effect of IS were 93.62% and 95.61%, respectively.

Linearity and lower limits of quantification. All the calibration curves were linear within the concentration range of 0.98–62.69 ng/mL for CCSG, 0.43–54.50 ng/mL for ononin, 0.92–59.06 ng/mL for calycosin, 4.29–274.52 ng/mL for DPPG and 3.91–250.50 ng/mL for DIFG in rat plasma with a correlation coefficient $r > 0.99$, which displayed good linear relationships. The regression equation, correlation coefficients and linear ranges for the five analytes were

Table 2. Extract recovery and matrix effect of the analytes in rat plasma ($n = 6$)

Analytes	Concentration (ng/mL)	Extraction recovery		Matrix effect	
		Mean (%)	RSD (%)	Mean (%)	RSD (%)
DPPG	68.64	76.38 ± 2.09	2.36	98.91 ± 2.13	3.32
	17.16	87.54 ± 4.01	4.72	98.27 ± 4.29	4.56
	4.290	92.48 ± 1.25	1.61	95.62 ± 2.54	2.72
Ononin	54.50	86.66 ± 2.48	2.60	99.21 ± 7.56	7.79
	13.64	104.71 ± 5.88	6.61	98.59 ± 2.69	2.86
	3.410	99.44 ± 1.66	2.13	98.58 ± 5.99	6.23
DIFG	62.64	79.39 ± 2.23	2.82	94.32 ± 3.19	3.27
	15.66	86.70 ± 2.77	3.52	96.29 ± 5.62	5.78
	3.915	81.38 ± 1.10	1.84	92.39 ± 6.19	6.24
Calycosin	59.08	92.35 ± 4.29	4.56	93.46 ± 4.96	5.10
	14.77	113.19 ± 8.92	8.35	91.39 ± 5.69	5.74
	3.693	105.69 ± 7.22	7.79	94.24 ± 1.34	1.66
CCSG	62.68	83.45 ± 3.27	3.86	95.28 ± 4.32	4.39
	15.67	84.70 ± 0.90	1.36	93.29 ± 4.29	4.41
	3.918	83.89 ± 3.99	3.46	96.46 ± 3.67	3.71
Theophylline	408.0	93.62 ± 1.21	1.59	95.61 ± 6.91	6.99

shown in Table 3. The average values of regression equation of the chromatographic profiles in rat plasma were: The LLOQ was 0.25, 0.43, 0.46, 0.54, and 0.49 ng/mL for CCSG, ononin, calycosin, DPPG, and DIFG, which were adequate sensitivity to pharmacokinetic studies of the content of these components in rats.

Accuracy and precision. The intra-day and inter-day precision and accuracy of the method were shown in Table 4. The intra-day and inter-day precisions derived from the QC samples ranged from 1.79–8.52% and 2.15–9.65%, respectively. The accuracies were all within -2.85–4.13% and -4.59–7.58.

Stability. As shown in Table 5, the short term, long-term, freeze-thaw, and auto sampler stability results indicated that CCSG, ononin, calycosin, DPPG, and DIFG were stable because all bias in concentrations were within -5.31–8.65%. Moreover, no significant difference for the five target compounds in rat plasma was found during different storage periods.

Table 3. The regression equation, correlation coefficients and linear ranges for the determination of five analytes in rat plasma

Analytes	Regression equation	R	Linear range (ng/mL)
CCSG	$y = 5.20 \times 10^{-3}x + 7.0 \times 10^{-3}$	0.9982	0.98–62.69
Ononin	$y = 6.76 \times 10^{-2}x + 3.4 \times 10^{-3}$	0.9992	0.43–54.50
Calycosin	$y = 3.00 \times 10^{-3}x - 7.0 \times 10^{-4}$	0.9992	0.92–59.06
DPPG	$y = 1.82 \times 10^{-2}x - 1.9 \times 10^{-3}$	0.9997	4.29–274.52
DIFG	$y = 5.40 \times 10^{-3}x - 6.3 \times 10^{-3}$	0.9987	3.91–250.5

Application of the analytical method in pharmacokinetics study

In this study, the above method was applied to the analyzed plasma samples obtained from rats administered orally with the *R. Astragali* extraction. The mean plasma concentration of CCSG, ononin, calycosin, DPPG, and DIFG ($n = 8$) versus time profiles were shown in Fig. 4, and the main pharmacokinetic parameters in rats were presented in Table 6. After the administration of ERA, the T_{max} of calycosin, DPPG, and DIFG was the same at 0.25 ± 0.12 h and the difference of $T_{1/2}$ at 2.98 ± 4.54 h, 4.27 ± 2.99 h, 8.05 ± 15.92 h. With T_{max} at 0.25 ± 0.12 h and $T_{1/2}$ at 2.98 ± 4.54 h, the absorption and scavenging rate of DPPG was the fastest among the five isoflavones. With T_{max} at 0.30 ± 0.14 h and $T_{1/2}$ at 10.90 ± 8.79 h, CCSG was absorbed and eliminated the slowest among five isoflavones. The $T_{1/2}$ parameters of flavonoid glycoside were faster than those of their corresponding aglucone by comparing CCSG to calycosin, and the values of C_{max} and AUC of the five isoflavones were approximately consistent with the contents of them in ERA. In addition, with the exception of calycosin, the other four compounds showed double peaks at 1.5 h. The phenomenon might be caused by three reasons, one reason was that the gastric emptying by a certain time, the drug was divided into two times to reach the small intestine causing the drug to enter the blood twice and display a bimodal. The second was that the drug absorptions in two different parts of the gastrointestinal tract were different, which led to abimodal. The next was circulation of the liver and intestine.

CONCLUSIONS

The method of UHPLC–MS/MS was established and validated to determine the CCSG, ononin, calycosin, DPPG, and

Table 4. Precision and accuracy of the analytes in rat plasma ($n = 6$)

Analytes	Concentration (ng/mL)	Intra-day		Inter-day	
		RSD (%)	RE (%)	RSD(%)	RE (%)
DPPG	68.64	6.620	−2.130	6.970	5.680
	17.16	7.190	−1.980	8.120	4.250
	4.290	5.360	2.310	5.120	2.640
Ononin	54.50	3.600	2.590	4.650	−1.030
	13.64	5.130	3.450	4.980	−2.010
	3.410	8.500	3.020	9.060	5.260
DIFG	62.64	5.820	−1.580	6.120	6.230
	15.66	8.520	−2.140	9.650	−4.590
	3.915	2.840	1.090	2.190	7.580
Calycosin	59.08	4.560	2.100	6.210	2.690
	14.77	5.350	2.310	4.380	5.320
	3.693	1.790	3.650	2.150	7.230
CCSG	62.68	1.860	−2.850	3.150	5.210
	15.67	3.600	2.110	6.790	6.980
	3.918	4.160	4.130	5.690	6.630
Theophylline	408.0	1.020	1.930	2.680	5.240

Table 5. Stability of the analytes in rat plasma under various storage conditions ($n = 6$)

Analytes	Concentration (ng/mL)	Short-term		Auto sample		Freeze-thaw		Long-term	
		RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)
DPPG	68.64	6.13	−2.09	5.29	3.19	6.92	−3.61	9.16	−3.64
	17.16	7.01	−1.91	4.68	−2.13	7.21	4.68	8.24	5.60
	4.290	4.39	2.12	4.98	2.51	5.96	5.63	7.68	−5.69
Ononin	54.50	3.59	2.38	4.19	3.96	5.42	3.66	8.62	−3.51
	13.64	5.19	3.41	3.51	2.54	6.38	5.14	5.98	8.65
	3.410	7.51	2.58	7.54	2.84	5.37	−5.31	7.81	−1.67
DIFG	62.64	5.41	−1.09	5.31	2.36	6.31	2.99	6.98	5.98
	15.66	7.68	−2.17	2.33	3.62	5.61	−1.38	8.51	4.21
	3.915	2.69	1.26	4.16	2.54	4.99	5.62	6.33	5.62
Calycosin	59.08	3.99	2.04	6.32	3.24	5.82	4.81	7.51	−3.34
	14.77	4.91	3.25	5.26	4.21	8.62	3.97	5.96	7.35
	3.693	1.67	3.39	4.25	2.61	7.59	5.23	8.26	5.16
CCSG	62.68	1.76	−2.56	1.93	6.21	8.21	4.19	7.51	4.83
	15.67	3.48	2.03	5.39	4.59	7.66	−3.33	4.99	4.36
	3.918	4.07	4.09	6.33	2.85	6.52	6.51	5.85	7.25
Theophylline	408.0	0.99	1.83	4.31	7.32	3.29	4.21	5.68	−2.31

Fig. 4. Mean plasma concentration-time profiles of DPPG, CCSG, ononin, calycosin, and DIFG in rats after oral administration of the whole plant of *Radix Astragali* extract ($n = 8$)Table 6. Pharmacokinetic parameters of the analytes after oral administration to rat plasma (mean $\pm$ SD, $n = 8$)

Parameters	DPPG	Ononin	DIFG	Calycosin	CCSG
Slope	-0.11 ± 0.09	0.00 ± 0.03	-0.12 ± 0.11	-0.06 ± 0.08	-0.06 ± 0.05
C_{max} (ng/mL)	160.78 ± 48.73	1.07 ± 0.12	120.97 ± 73.02	19.60 ± 3.99	39.79 ± 8.57
T_{max} (h)	0.25 ± 0.12	0.27 ± 0.10	0.25 ± 0.12	0.25 ± 0.12	0.30 ± 0.14
K_e	0.25 ± 0.21	0.00 ± 0.08	0.29 ± 0.25	0.15 ± 0.18	0.13 ± 0.11
$T_{1/2}$ (h)	2.98 ± 4.54	4.89 ± 11.83	4.27 ± 2.99	8.05 ± 15.92	10.90 ± 8.79
AUC_{0-t} (ng h/mL)	159.67 ± 47.75	1.24 ± 0.26	120.92 ± 70.15	36.98 ± 7.27	57.09 ± 10.36
$AUC_{0-\infty}$ (ng h/mL)	167.80 ± 62.97	4.28 ± 5.04	150.30 ± 62.52	58.94 ± 43.92	113.49 ± 36.34
$AUC_{t-\infty}$ (ng h/mL)	8.13 ± 46.77	3.04 ± 5.13	29.38 ± 26.25	21.96 ± 49.05	56.40 ± 42.12

DIFG simultaneously in rat plasma after oral administration of ERA and investigated on their pharmacokinetic studies following a simple plasma preparation procedure and a shorter running time. The study provided adequate extraction recovery which has great accuracy and precision. The method has higher selectivity and stability than that of previous methods. This is the first time to the pharmacokinetic profiles of the five isoflavones in rat plasma after oral administration of ERA. The pharmacokinetics results are helpful to understand the biotransformation process of

flavonoids glycosides to aglucones in vivo and will provide some valuable reference to clinical application of this herb.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

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